PARTIAL RESOLUTION OF ENERGY-LINKED REACTIONS IN RHODOSPIRILLUM RUBRUM CHROMATOPHORES *

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1. Introduction

Chromatophores isolated from *Rhodospirillum rubrum* contain in addition to their photophosphorylation mechanism a number of the energy-linked functions found in rat liver and ox-heart submitochondrial particles. Like submitochondrial particles, the chromatophores catalyze an electron transport coupled phosphorylation of ADP to ATP [1], energy-linked ion transport [2], the energy dependent reduction of NAD^+ by succinate [3], and an energy-linked transhydrogenation between NADH and $NADP^+$ [4]. In addition, chromatophores are able to couple the synthesis of pyrophosphate to electron transport [5].

Chromatophores further resemble submitochondrial particles in that they contain ATPase activity. The ATPase activity has been postulated to be a result of a reversal of the phosphorylation mechanism and the hydrolytic cleavage of an energized intermediate [6]. Studies of the coupling factor, F_1, from ox-heart mitochondria suggested that mitochondrial ATPase represents the terminal enzyme of the phosphorylation mechanism [7]. In analogy with the mitochondrial and chromatophore ATPase activity, the inorganic pyrophosphatase activity of *R. rubrum* has been postulated as being due to a reversal of a pyrophosphate synthesizing reaction [5].

The question arises as to whether one or two terminal enzymes catalyze the phosphorylation of P_i (to inorganic pyrophosphate) and ADP (to ATP) in *R. rubrum*.

Boyer [8] pointed out that the slow rate of pyrophosphate synthesis may result from the substitution of a phosphate for ADP at an enzymatic site identical to that utilized for ATP synthesis. On the other hand Baltscheffsky and Von Stedingk [5] have proposed that pyrophosphate synthesis represents a side path of the reaction sequence leading to ATP synthesis.

This communication reports on the preparation of *R. rubrum* particles resolved with respect to the energy-linked transhydrogenase and deficient in ATPase or pyrophosphatase activity. Our results obtained with these particles suggest that the phosphorylation leading to pyrophosphate and adenosine triphosphate synthesis is catalyzed by two distinct enzymes.

Abbreviations

C_T *R. rubrum* chromatophores depleted of transhydrogenase factor

C_{AT} *R. rubrum* chromatophores depleted of transhydrogenase factor and lacking adenosine triphosphatase activity

C_{PT} *R. rubrum* chromatophores depleted of transhydrogenase factor and lacking inorganic pyrophosphatase activity

TH_1 transhydrogenase factor

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2. Experimental procedure

Chromatophores were prepared from photosynthetically grown *R. rubrum* (Van Niel Strain –1) [9] by grinding with sand [10]. Bacteriochlorophyll was assayed according to the method of Clayton [11]. Incorporation of $^{32}P_i$ into glucose-6-phosphate was measured by the isobutanol extraction procedure of Lindberg and Ernster [12]. Energy-linked transhydrogenation was assayed by the method of Keister and Yike [4] and ATPase activity was measured as previously reported [13]. The transhydrogenase factor (TH_1) was prepared as described earlier [14].

C_T particles were prepared by washing chromatophores with 0.1 M Tris-HCl buffer–10% sucrose, pH 8 until they were free of measurable energy-linked transhydrogenase activity [14].

C_{AT} particles were prepared by adding 3 ml of C_T particles (320 μg bacteriochlorophyll per ml) suspended in 0.1 M glycylglycine–10% sucrose, pH 8 to 50 ml of a solution containing 0.25 M glycylglycine and 2 M LiCl, pH 8. After continuous stirring at 4° for 10 min the suspension was centrifuged 30 min at 50,000 rpm in a Spinco 50 rotor. The sedimented chromatophores were resuspended in 0.10 M glycylglycine–10% sucrose, pH 8 to a concentration of 300 μg of bacteriochlorophyll per ml.

C_{PT} particles were prepared by the dropwise addition of 1-butanol to C_T particles (275 μg bacteriochlorophyll per ml) suspended in 0.2 M glycylglycine buffer, pH 7.8. After the 1-butanol concentration had reached 3.1% the suspension was stirred for an additional 10 min at 4° and then centrifuged for 1 hr at 40,000 rpm in a Spinco 40 rotor. The chromatophore pellet was resuspended in a 0.2 M glycylglycine buffer, pH 7.8, and dialyzed at 4° against the same buffer for eleven hours.

3. Results

The energy-linked transhydrogenase of *R. rubrum* chromatophores driven by light, ATP or pyrophosphate has been resolved into a soluble (TH_1) and a particulate (C_T) component [14]. Little or no energy-linked transhydrogenase is demonstrated in the particulate fraction in the absence of TH_1. Addition of TH_1 (table 1) totally reconstitutes transhydrogenation driven by the three energy sources (the pyrophosphate driven reaction is not shown).

Table 1
Reconstitution of the energy dependent transhydrogenation of C_T by TH_1.

Additions	Transhydrogenase activity	
	Energy source	
	Light	ATP
Chromatophores	105	39.5
C_T	1.4	4.4
C_T plus TH_1	103	36.3

The reaction medium (3 ml) at 23° contained 44 mM glycylglycine buffer pH 8, 125 mM sucrose, 180 mM ethanol, 0.067 mM NADH, 0.334 mM $NADP^+$, 2.67 mM $MgCl_2$, 175 units of yeast alcohol dehydrogenase and chromatophores (20 μg bacteriochlorophyll), 0.85 mM ATP. Illumination was 7.5×10^4 erg cm^{-2} sec^{-1}. When added 2.75 mg TH_1 was sufficient to stimulate maximally the transhydrogenase activity. The activity is given as μmoles of $NADP^+$ reduced per mg bacteriochlorophyll per hr measured as an increase in OD at 340 mμ [14].

Table 2
Effect of extraction of C_T with 1-butanol on ATPase, pyrophosphatase and photophosphorylation.

Particle preparation	Enzymatic activity		
	ATPase	Pyrophosphatase	Photophosphorylation
C_T	348	158	313
C_{PT}	356	11	261

Photophosphorylation was assayed at 30°C in a reaction medium (3 ml) containing 28 mM glycylglycine buffer pH 7.8, 3.3 mM $^{32}P_i$ (3×10^6 cpm/μmole P_i), 10 mM $MgCl_2$, 3.3 mM ATP, 50 mM glucose, 0.4 mg yeast hexokinase, 0.44 mM phenazine methosulfate, C_T and C_{PT} chromatophores (44 μg bacteriochlorophyll). The reaction was terminated at 5 min with perchloric acid [13] and the ATP synthesized evaluated according to the procedure of Lindberg and Ernster [12]. Light illumination was 7.5×10^4 erg cm^{-2} sec^{-1}. Photophosphorylation activity is expressed as μmoles ATP synthesized per mg bacteriochlorophyll per hr, ATPase and pyrophosphatase activity as μmoles of substrate hydrolyzed per mg bacteriochlorophyll per hr.

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Table 3
Effect of extraction of C_T with 1.9 M LiCl on ATPase and pyrophosphatase.

Particle preparation	Enzymatic activity	
	ATPase	Pyrophosphatase
C_T	276	226
C_{AT}	14	224

ATPase was assayed for 10 min at 30° in a reaction medium (1 ml) containing 33 mM glycylglycine buffer pH 8, 96 mM sucrose, 2 mM ATP, 2 mM $MgCl_2$, 0.005 mM carbonylcyanide-*m*-chlorophenylhydrazone, 3 mM phosphoenolpyruvate, 0.03 mg pyruvate kinase (Boehringer), and resolved chromatophores (30 μg bacteriochlorophyll).
Pyrophosphatase activity was assayed for 5 min at 30° in a reaction medium (1 ml) containing 42 mM glycylglycine buffer pH 8, 123 mM sucrose, 5 mM $MgCl_2$, 5 mM sodium pyrophosphate, 0.005 mM carbonylcyanide-*m*-chlorophenyldrazone and resolved chromatophores (30 μg bacteriochlorophyll). Activities are given as μmoles of substrate hydrolyzed per mg bacteriochlorophyll per hr.

Table 4
Reconstitution of energy dependent transhydrogenation in C_T and C_{AT}.

Additions	Transhydrogenation activity		
	Energy source		
	Light	ATP	Pyrophosphate
C_T	115	38	26
C_T + 20 μg oligomycin	119	2	22
C_{AT}	34	0	8
C_{AT} + 20 μg oligomycin	83	0	18

Transhydrogenation was assayed as in table 1. When added, sodium pyrophosphate was 1.7 mM.

hydrogenase depleted particles (C_T) as well as their photophosphorylation is comparable to the activities of unresolved particles (table 2). When C_T was extracted with 3.1% of 1-butanol the resulting particles (C_{PT}) became deficient in pyrophosphatase activity. Photophosphorylation of ADP was only slightly lower in C_{PT} than in C_T. On the other hand, extraction of C_T with 1.9 M LiCl results in chromatophore preparations (C_{AT}) which have a low level of ATPase activity but retain a level of pyrophosphatase activity identical to that found in the nonextracted chromatophores (table 3).

Reconstituted energy-linked transhydrogenation was used to evaluate the ability of light, ATP and pyrophosphate to drive this reaction in C_T and C_{AT}. As shown in table 4, C_{AT} particles in the presence of TH_1 catalyze the energy linked transhydrogenation driven by light or pyrophosphate, but not by ATP. C_{PT} particles (data not shown) in the presence of TH_1 catalyze the energy-linked transhydrogenation driven by light. Keister and Yike [4] have demonstrated that ATP-driven transhydrogenation in chromatophores unresolved with respect to transhydrogenase activity is inhibited by oligomycin. From table 4 it can be seen that oligomycin inhibited reconstituted transhydrogenation driven by ATP in C_T particles but had little influence on the reaction driven by light or pyrophosphate. Following LiCl extraction of C_T, oligomycin stimulated the pyrophosphate and light driven reactions by almost 250%. The action of oligomycin on transhydrogenation in C_{AT} is similar to that observed with non-phosphorylating submitochondrial particles [16]. As postulated for the submitochondrial system this stimulation may be related to the inhibition of an energy dissipating reaction. Results identical to those obtained with LiCl extraction of C_T were obtained when the transhydrogenase deficient particles were extracted with 3 M urea.

4. Discussion

Although extraction of chromatophores with 1.9 M LiCl results in the loss of ATPase activity as well as the ability of the particles to photophosphorylate ADP, it is uncertain as to whether the ATPase is detached from the particles or inactivated while still bound to the membrane. MacLennan and his associates [17] have reported that extraction of submitochondrial particles with NaCl selectively solubilizes inactive subunits of mitochondrial ATPase from the membrane.

Electron microscopic observations of negatively stained preparations have not revealed any major morphological difference between control chromatophores and the LiCl treated preparations.

Although inner membrane spheres were seen in some fields as previously reported by Low and Afze-

lius [18] they were not visible in most of the chromatophores.

The specific loss of pyrophosphatase activity upon 1-butanol extraction and of ATPase activity on extraction with 1.9 M LiCl suggests that these two activities reside in different enzymes. The ability of pyrophosphate to drive the energy-linked transhydrogenation in C_{AT} at an unimpaired rate relative to that of the light driven reaction while the ATP driven reaction is completely inhibited is shown in table 4. This observation indicates that the enzyme system (s) required for the formation of energized intermediates from light or pyrophosphate are still functional. It is obvious that the pathway for synthesis of energized intermediates from ATP is inoperative in these particles. In addition, pyrophosphate but not ATP synthesis is observed in C_{AT} [19].

It is concluded that the light dependent phosphorylation reactions leading to the synthesis of ATP and pyrophosphate in chromatophores of *R. rubrum* represent enzyme systems which can be at least partially separated and are not totally interdependent.

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